Stability of Carotene and Vitamin A in Dry Mixtures

MONROE E. WALL AND EDWARD G. KELLEY
Eastern Regional Research Laboratory, Philadelphia 18, Pa.

The research reported in this paper was designed to study factors affecting the stability of carotene and vitamin A in dry mixtures. It was hoped that the information thus secured would be of assistance to mixers of poultry and animal feed.

It was found that temperature, concentration, carrier, and vitamin source markedly affected the stability of carotene and vitamin A ester preparations. Stability increased with decreasing temperatures and with increasing concentrations. Carotene preparations were more stable than corresponding vitamin A ester mixtures. A carotene preparation prepared by molecular distillation of broccoli leaf meal extracts had marked stability even at room temperature.

Findings show that feed mixers can prepare relatively stable dry mixtures of high vitamin A potency by using a proper combination of temperature, concentration, carrier, and vitamin source.

THE retention of carotene and vitamin A in dry mixtures is a matter of economic importance to producers and users of mixed feedstuffs. Although the stability of carotene in leaf meals and of carotene and vitamin A in oil solution has been studied by many workers, their stability in feed mixtures has received limited attention.

The object of this investigation was to determine the stability of a number of carotene and vitamin A preparations mixed with several dry carriers and kept under varying storage conditions.

MATERIALS AND METHODS

VITAMIN CONCENTRATES. A description of the carotene and vitamin A products used in this investigation is given in Table I. The alfalfa and carrot carotene concentrates were obtained through the courtesy of the Nutritional Research Associates, Inc., the vitamin A feeding oil from National Oil Products Co., and the distilled vitamin A concentrate from Distillation Products, Inc. Most of the vitamin A preparations were in the ester form, and all references to vitamin A in the paper refer to the ester form only. The carotene concentrates from broccoli were prepared at this laboratory. The concentrates were analyzed for tocopherol and the data are shown in Table I. Although the tocopherol contents of the various products varied widely, there is little evidence that tocopherol had any effect on stability under the experimental conditions.

CARRIERS. Three products of actual or potential interest as feedstuffs were used as carriers for the various vitamin preparations: a poultry feeding mash (7), hexane-extracted soybean meal, and hexane-extracted broccoli leaf meal. The chief constituents of the mash were white corn meal (17%), ground oats (15%), wheat bran (15%), ground wheat (23%), soybean oil meal (20%), meat scraps (5%), steamed bone meal (1.0%), oyster shell flour (1.5%), and salt mix (0.5%). Vitamin A and carotene contents of the mash were negligible. The ground, whole soybean and broccoli leaf meals were exhaustively extracted with hexane, and consequently contained no carotene.

PREPARATION AND STORAGE OF DRY MIXTURES. The various concentrates were analyzed for carotene and vitamin A ester and α-tocopherol by the methods of Wall and Kelley (*10, 12, 13*). An appropriate quantity of concentrate was dissolved in 50 ml.

TABLE I. DESCRIPTION OF CAROTENE AND VITAMIN A CONCENTRATES

Concentrate	Source	Method of Preparation	Vitamin A Equivalent,[a] I.U./Gram	Tocopherol, Mg./Gram	Carrier Oil
Carotene	Alfalfa leaf meal	Saponification of leaf extract and recovery of unsaponifiable fraction	2,430	2.2	Crude soybean
Carotene	Carrot meal	Solvent extraction of carrots	2,760	2.3	Crude soybean
Carotene	Broccoli leaf meal	Solvent extraction of leaf meal followed by removal of phospholipide and molecular distillation at 200-220° C.	16,300	20.0	Refined cottonseed
Carotene	Broccoli leaf meal	Same as above. Product is residual oil remaining after molecular distillation at 200-220° C.	7,100	1.0	Refined cottonseed
Carotene	Broccoli leaf meal	Same as above. Product is undistilled oil solution	8,000	5.0	Refined cottonseed
Vitamin A (ester form)	Fish liver		3,000	0.0	Fish liver
Vitamin A (ester form)	Fish liver	Molecular distillation of fish liver oils	300,000	...	Fish liver and vegetable

[a] Vitamin A equivalent for carotene calculated by multiplying carotene content in micrograms per gram by factor 1.67.

of hexane, and the resultant solution was thoroughly mixed with 100 grams of carrier by means of a mortar and pestle. The solvent was then removed in a vacuum oven at a temperature of 35° to 40° C. The final vitamin concentrations, calculated as vitamin A, were 3000 and 300,000 I.U. per pound. It was necessary that the most potent dry mixtures be prepared from concentrated vitamin sources. It was impossible to prepare concentrated free-flowing, dry mixtures from low potency vitamin-containing oils because of the necessity of adding large amounts of oil to the dry carriers.

The samples were stored in 1-pound, kraft, standard weight paper bags, a procedure recommended by Silker (*6*) as best simulating large scale storage conditions in burlap bags. The storage temperatures were 4°, 25°, and 37.5° C. In some instances only the 25° temperature was tested. The samples were analyzed for carotene or vitamin A initially and at 1-month (30-day) intervals for a maximum of 6 to 8 months until 50% or more of the carotene or vitamin A was destroyed.

CALCULATION OF RESULTS. When the logarithm of the carotene or vitamin A concentration was plotted against the storage time, the plot approximated a straight line; hence, the data could be represented as a first-order process. All data were consequently plotted on semilog paper; the carotene or vitamin A concentrations were shown in terms of percentage retention and the storage time in months. The method of least squares was then applied to the data to obtain the ideal straight-line equations. The velocity constant, k, for the decomposition of carotene or vitamin A in storage was calculated by multiplying the negative slopes of the various straight lines by 2.303. The half-life (time necessary for half the carotene or vitamin A to decompose) was calculated by the formula $t^{0.5} = \frac{0.693}{k}$.

The plot of log k against the reciprocal of the absolute temperature also approximated a straight line and was corrected by the method of least squares.

The decomposition of carotene and vitamin A in mixed feeds is probably far more complex than is implied by a first-order reaction. However, the over-all course of the reaction seems to fit this generalization, and enables one to compare the various storage experiments in simple mathematical terms. Legault *et al.* (*3*) have used this technique in their treatment of sulfide disappearance in stored, dehydrated vegetables.

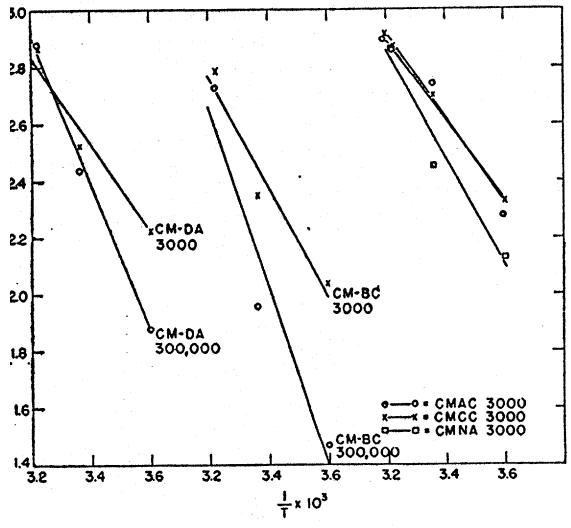

Figure 1. Effects of Temperature and Concentration on Stability of Molecularly Distilled Carotene Mixed in Chick Mash

RESULTS AND DISCUSSION

Because it is necessary to discuss some forty different storage experiments, it is convenient to give each experiment a code name. The code is shown in Table II. In use the carrier is named first, followed by the vitamin source, the concentration, and the temperature. For example, CM-BC-300,000-4° is chick mash in which molecularly distilled broccoli carotene has been mixed to give a concentration of 300,000 I.U. per pound and stored at 4° C.

Figures 1 and 2 show a few typical plots on semilog paper of per cent carotene or vitamin A retention against time. Figure 3 presents the plots of the logarithm of the reaction constants against the reciprocal of the absolute temperature. The fact that all these graphs approximate straight lines is the basis for considering the over-all course of the destruction of carotene or vitamin A to be a first-order process.

Table III gives the essential data for all the experiments. The storage series are presented in the order of decreasing velocity constants or increasing half-life periods. In addition, the equations of the corrected straight-line graph of each experiment are included.

A study of the data reveals the fact that many factors affect the stability of carotene or vitamin A mixed on dry carriers. Although in most instances the stability of the various preparations

TABLE II. EXPLANATION OF CODE FOR STORAGE EXPERIMENTS

Code	Description
AC	Alfalfa carotene concentrate
CC	Carrot carotene concentrate
BC	Broccoli carotene concentrate produced by molecular distillation
BCy	Broccoli carotene concentrate residue after molecular distillation
BCx	Broccoli carotene concentrate undistilled
NA	Vitamin A feeding oil
DA	Vitamin A produced by molecular distillation
CM	Chick mash
S	Extracted soybean meal
B	Extracted broccoli leaf mea

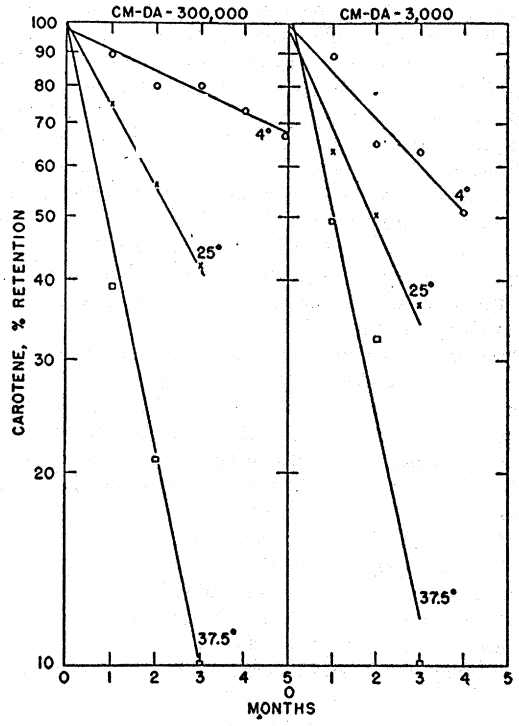

Figure 2. Effects of Temperature and Concentration on Stability of Molecularly Distilled Vitamin A Mixed in Chick Mash

is the resultant of the interaction of all the factors concerned, it is possible and desirable to discuss them separately.

TEMPERATURE. This was undoubtedly the most important variable. Table III shows that in most cases temperature outweighed all the other factors combined. Carotene or vitamin A stability varied inversely with the temperature, as indicated by decreasing reaction rates and increasing half-life periods as the temperature was decreased. Similar results have been reported in studies of the stability of carotene in oil solution and on solid carriers (*5*, *11*).

From a practical standpoint, the data in Table III show that although it was possible to make certain preparations which were stable at 25° C. (77° F.) (samples 35, 37, 38, 39, and 40), it was impossible under experimental conditions to prepare dry mixtures which were stable at 37.5° C. (98° F.) (samples 1, 2, 3, 5, 6, 7, and 9). Probably storage of the average mixed feedstuff at extreme summer temperatures would result in loss of over half the carotene or vitamin A in 1 month. On the other hand, refrigerated storage would result in a marked improvement in the stability of samples which under other conditions rapidly lose their vitamin A content (compare samples 30, 33, and 34 with samples 14, 18, and 22).

CONCENTRATION. In almost all cases studied, the carotene and vitamin A samples containing 300,000 I.U. per pound were more stable than the corresponding 3000 I.U. preparations (compare samples 15 and 26, 14 and 20, 23 and 40, 22 and 35, 31, and 39, 30 and 36, 34 and 41). The differences in stability between the two concentrations were markedly influenced by temperature; the lower the temperature the greater the differences in stability. At 37.5° C. the preparations were all so unstable that concentration had no effect (compare samples 1 and 5 and 7 and 9). Similar results were found, whether the 3000 I.U. samples were prepared by mixing the appropriate quantity of vitamin concentrate with the dry carrier or by dilution of the 300,000 I.U. preparations with more carrier.

The finding that increased carotene or vitamin A concentration leads to greater stability in dry samples is in marked contrast with results obtained by Wall and Kelley with carotene in oil samples (*11*). They found little difference in samples containing the equivalent of 70,000 and 700,000 I.U. per pound, but preparations with 3,500,000 and 10,000,000 units per pound were extremely unstable. It is possible, of course, that concentrations much lower than 70,000 I.U. would have shown lowered stability.

As shown in previous literature, the situation with dry samples is somewhat confused. Bickoff and Williams (*2*) working with pelleted samples found a progressive decrease in stability when the carotene concentration (crystalline carotene) was progressively increased from approximately 150,000 to 750,000 I.U. per pound. These workers used a storage temperature of 37° C. At room temperature, probably about 25° C., Morgal *et al.* (*5*) reported only 4 to 19% destruction in 5 months when an alfalfa carotene concentrate was mixed with a soybean meal base in a concentration of 60,000 I.U. per pound. Under similar conditions Mitchell *et al.* (*4*) working with a concentration of 700,000 I.U. per pound found 60% losses. Similar experiments in the present investigation with distilled broccoli carotene (samples 23 and 40) showed losses of 67% for the 3000 I.U. samples and 17% for the 300,000 I.U. preparation in 5 months. As is shown subsequently, it is probable that an undistilled broccoli carotene preparation would have lost considerably more.

It is conceivable that stability of carotene goes through a minimum-maximum curve with varying concentration. It would be low at 3000 I.U. per pound (4 micrograms per gram), rise to a maximum somewhere between 50,000 and 300,000 I.U. per pound (66 to 400 micrograms per gram), and then taper off as the concentration is increased. Such a sequence can be explained logically if one assumes that loss of carotene in dry mixtures de-

TABLE III. REACTION RATES, HALF-LIFE VALUES, AND STRAIGHT-LINE EQUATIONS SHOWING RATE OF DECOMPOSITION OF CAROTENE AND VITAMIN A PREPARATIONS MIXED WITH DRY CARRIERS

Sample No.	Preparation	Reaction Rate $K \times 10^4$, months^{-1}	Half-Life, Months	Straight-Line Equation[a]
1	CM-DA-300,000-37.5°	760	0.91	$y = 1.98 - 0.330x$
2	CM-NA-3000-37.5°	745	0.93	$y = 2.05 - 0.324x$
3	CM-CC-3000-37.5°	740	0.94	$y = 2.05 - 0.322x$
4	B-DA-3000-25°	736	0.94	$y = 1.96 - 0.320x$
5	CM-DA-3000-37.5°	736	0.94	$y = 2.03 - 0.320x$
6	CM-AC-3000-37.5°	727	0.95	$y = 2.04 - 0.316x$
7	CM-BC-3000-37.5°	617	1.11	$y = 2.03 - 0.268x$
8	CM-AC-3000-25°	548	1.25	$y = 2.05 - 0.238x$
9	CM-BC-300,000-37.5°	543	1.27	$y = 2.03 - 0.236x$
10	B-NA-3000-25°	530	1.30	$y = 1.96 - 0.230x$
11	CM-CC-3000-25°	503	1.37	$y = 2.09 - 0.218x$
12	B-DA-300,000-25°	503	1.37	$y = 1.99 - 0.218x$
13	S-CC-3000-25°	480	1.44	$y = 2.03 - 0.208x$
14	CM-DA-3000-25°	332	2.09	$y = 1.98 - 0.144x$
15	S-DA-3000-25°	322	2.14	$y = 1.98 - 0.140x$
16	CM-BCx-3000-25°	320	2.17	$y = 2.08 - 0.139x$
17	CM-BCy-3000-25°	288	2.40	$y = 2.06 - 0.125x$
18	CM-NA-3000-25°	286	2.42	$y = 2.04 - 0.124x$
19	S-NA-3000-25°	276	2.51	$y = 2.02 - 0.120x$
20	CM-DA-300,000-25°	276	2.51	$y = 1.99 - 0.120x$
21	CM-BCx-300,000-25°	270	2.56	$y = 2.05 - 0.117x$
22	CM-BC-3000-25°	226	3.05	$y = 2.05 - 0.098x$
23	S-BC-3000-25°	216	3.20	$y = 1.99 - 0.094x$
24	CM-CC-3000-4°	214	3.23	$y = 2.04 - 0.093x$
25	B-CC-3000-25°	207	3.34	$y = 2.02 - 0.090x$
26	S-DA-300,000-25°	198	3.50	$y = 1.98 - 0.086x$
27	CM-AC-3000-4°	191	3.62	$y = 2.04 - 0.083x$
28	B-AC-3000-25°	177	3.90	$y = 2.04 - 0.077x$
29	S-AC-3000-25°	177	3.90	$y = 1.97 - 0.077x$
30	CM-DA-3000-4°	166	4.17	$y = 2.00 - 0.072x$
31	B-BC-3000-25°	154	4.50	$y = 2.01 - 0.067x$
32	B-BCx-300,000-25°	134	5.15	$y = 2.01 - 0.058x$
33	CM-NA-3000-4°	134	5.15	$y = 2.00 - 0.058x$
34	CM-BC-3000-4°	110	6.30	$y = 2.03 - 0.048x$
35	CM-BC-300,000-25°	92	7.50	$y = 2.01 - 0.040x$
36	CM-DA-300,000-4°	76	9.10[b]	$y = 1.98 - 0.033x$
37	CM-BCy-300,000-25°	74	9.35[b]	$y = 1.99 - 0.032x$
38	B-BCy-300,000-25°	71	9.70[b]	$y = 2.00 - 0.31x$
39	B-BC-300,000-25°	53	13.0[b]	$y = 1.99 - 0.023x$
40	S-BC-300,000-25°	30	23.1[b]	$y = 1.98 - 0.013x$
41	CM-BC-300,000-4°	30	23.1[b]	$y = 2.00 - 0.013x$

[a] y = log carotene concentration (% retention); x = time in months (30 days).

[b] Half-life values, calculated by extrapolation from data obtained from experiments of 6-8 months' duration. While there is no doubt that these preparations are highly stable, half-life periods of extreme length found by extrapolation such as those found for samples 39, 40, and 41 cannot be regarded as highly accurate.

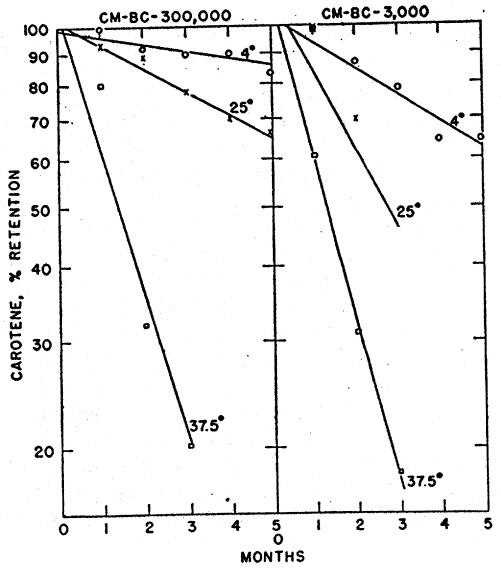

Figure 3. Effects of Temperature on Reaction Rate Constants of Carotene and Vitamin A Mixed with Chick Mash

pends on the surface area exposed. Dilute preparations would have a relatively large area exposed to oxidation. Furthermore, when the concentration is too high, peroxides may accumulate which could cause rapid breakdown of the carotene. From the data it seems that a similar case could be made for the oxidation of vitamin A in dry mixtures.

From a practical viewpoint the results shown in Table III indicate that samples with concentrations of 3000 I.U. per pound (which are recommended potencies for most chick mashes) have stabilities 50 to 500% less than corresponding samples with potencies of 300,000 I.U. Hence it would seem desirable to keep carotene or vitamin A in a concentrated refrigerated premix as long as possible before mixing into feed mashes at 3000 I.U. per pound.

Nature of Carrier. The stabilities of the various vitamin A and carotene preparations varied greatly with the carrier used. Extracted soybean meal was an excellent vehicle for both vitamin A and carotene (Table III, samples 26 and 40). Extracted broccoli leaf meal was an excellent carrier for carotene but poor for vitamin A (compare samples 13 and 39). Chick mash was generally inferior for both carotene and vitamin A (compare samples 20 and 35).

There is no good explanation for these effects of different carriers on carotene and vitamin A stability. Mitchell *et al.* (*4*) have shown that destruction of carotene was much greater on sucrose or starch than on soybean or cottonseed meals. They suggest that lack of antioxidants in the refined carriers is the cause of poor stability. On the other hand, the present experiments have shown that solvent-extracted soybean meal is an excellent carrier for carotene or vitamin A, whereas chick mash is inferior. The former is low in fat-soluble antioxidants; the latter is a mixture of crude and unrefined products.

At present, therefore, the choice of solid carriers for carotene or vitamin A must be largely empirical. Of the products tested, soybean meal is an excellent carrier, and broccoli gives good results with carotene.

Vitamin Source. The effects of this factor on stability were overshadowed by the other environmental conditions previously discussed. Comparing the carotene and vitamin A samples separately, one can say that in general the carotene group is more stable—compare, for example, samples 15, 19, and 26 with samples 23, 29, and 40. However, temperature, concentration, and carrier all influence the magnitude of the differences in stability—for example, as the temperature is lowered or raised from 25° C. the differences in stability between carotene and vitamin A decrease (compare samples 20 and 35 with samples 36 and 41, 1 and 9). Much greater differences between the carotene and vitamin A groups are found at 300,000 I.U. than at 3000 I.U. per pound (compare 26 and 40 with 15 and 23) and with broccoli leaf meal as a carrier, carotene is much more stable at all concentrations (compare 31 and 39, 4 and 12). Moreover, distilled carotene is more stable than untreated carotene, and hence distilled preparations will show up more favorably when compared with vitamin A. In spite of all these complications, the trend of increased carotene stability over that of vitamin A ester seems well established in the authors' experiments.

Turning to the individual groups, little difference in stability was noted between the two vitamin A preparations. Considerable variations were found when the stability of different carotene preparations was compared. At the 3000 I.U. level where comparison was possible, carotene from carrots was definitely less stable than that from alfalfa and broccoli (compare 13, 24, and 25 with 23, 29, 28, 31, 27, and 34). One explanation may lie in the fact that carotene from carrots contains a larger proportion of α-carotene than preparations from leaf sources; however, there is no proof that α-carotene is less stable than the beta form. As shown previously, the other experimental factors involved tend to obscure individual carotene effects.

Examination of the data does reveal one very clear-cut and important point. It will be noted from Table III that the various distilled broccoli carotene preparations containing 300,000 I.U. were extraordinarily stable at room temperature (compare samples 35, 37, 38, 39, and 40) with half-lives of 7.5 to 23 months. These distilled materials were prepared by hexane extraction of broccoli leaf meal; the extract was freed of phospholipides, evaporated, dissolved in cottonseed oil, and then molecularly distilled at temperatures up to 220° C. (*9*). A corresponding sample prepared in an identical manner with the exception that the distillation step was omitted had a half-life of only 2.56 months (sample 21). This result was so unexpected that the experiment was repeated twice, starting with different batches of broccoli leaf meal. Qualitatively similar results were obtained each time; the undistilled extract was much less stable than the distilled preparation. It seems evident that the increased stability of the distilled carotene preparations was due not to any unique stabilizing factors found only in broccoli leaf meal but to the distillation process.

A study of the effect of molecular distillation on carotene is now in process. Some preliminary results are of interest. The carotene distillates were partially purified by chromatographic adsorption on activated magnesia by the method of Wall and Kelley (*12*). The homogeneous carotene band thus obtained was examined spectroscopically by the method of Beadle and Zscheile (*1*). It was found that carotene distilled at 200° to 220° C. contained only 10 to 15% of all-trans β-carotene. According to Zechmeister (*14*), the remaining carotene is presumably a mixture of neo-β-carotene isomers formed by heating during the distillation period. Extracts subjected to milder heating at 140° to 160° C., contained 30 to 40% of all-trans β-carotene; and undistilled extracts contained 60 to 70% of all-trans β-carotene. Although the method of Beadle and Zscheile is not absolutely accurate for the complex mixture of isomers which are likely to be present in heated extracts (*14*), it seems probable that the observed trend of increased isomerization associated with increased distillation temperature is correct.

From these facts it is probable that the carotene prepared by molecular distillation consists largely of isomers which are more stable than the normal all-trans β-carotene. Since the distilled carotene is designed for use in poultry feeds, a brief statement of its biological potency may be appropriate. Experiments conducted at the University of Delaware have shown (*7*) that the dis-

tilled extracts on a calculated unitage basis (1 microgram of carotene = 1.66 I.U. of vitamin A) are as potent for broilers as carotene in broccoli leaf meal and vitamin A feeding oil over a range of 500 to 3000 I.U. per pound, and as potent as vitamin A for egg production at 3000 I.U. per pound (*8*).

SUMMARY

A number of carotene and vitamin A concentrates were mixed with solid carriers. Loss of carotene or vitamin A during storage followed the over-all pattern of a first-order reaction. By plotting the logarithm of the carotene concentration against the storage time, the reaction rate constants and time for 50% loss were calculated. These constants provide a simple mathematical means for comparing a large number of storage experiments.

The following factors play an important role in the stability of dry carotene or vitamin A mixtures:

TEMPERATURE. Decreasing storage temperature increased stability.

CONCENTRATION. A 100-fold increase in concentration from 3000 to 300,000 I.U. per pound resulted in marked increase in stability.

CARRIER. The effects of this factor were often obscured by other variables. Extracted soybean meal afforded highest stability; extracted broccoli leaf meal was excellent for carotene but poor for vitamin A; chick mash was inferior.

VITAMIN SOURCE. Effects of this factor were often overshadowed by the other environmental conditions. When a stable carrier was used, there were no significant differences between the vitamin A and carotene preparations at the 3000 I.U. level. At 300,000 I.U., the molecularly distilled carotene from broccoli showed a marked increase in stability over corresponding vitamin A or undistilled carotene preparations. The distilled carotene consists largely of neo-β-carotene isomers. The carotene thus prepared has been shown by Skoglund *et al.* (*7–8*) to have full biological potency for poultry.

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